

July 2, 2013

**Re: Statement regarding Land-based Tests of the Siemens Water Technologies LLC (USA)
SeaCURE Ballast Water Management System**

To Whom It May Concern:

This is to confirm that the land-based tests of the SeaCURE ballast water management system developed by Siemens Water Technologies LLC, Union, NJ 07083, USA as documented in

Final Report, Land-based Evaluation of the Siemens Water Technologies SiCURE™ Ballast Water Management System, September 2010 (MERC ER02-10)

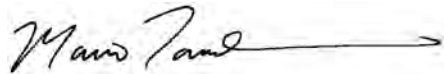
were undertaken according to

Test Plan for the Performance Evaluation of the Siemens SiCURE Ballast Water Management System, May 2009

which was evaluated and approved by the Bundesamt für Seeschifffahrt und Hydrographie (BSH), Hamburg prior to the tests.

Please let me know if you have any further questions.

Kind Regards,



Dr. Mario N. Tamburri
Director, Maritime Environmental Resource Center
Professor, University of Maryland Center for Environmental Science
+1-410-326-7440
tamburri@umces.edu



Land-Based Evaluations of the Siemens Water Technologies SiCURE™ Ballast Water Management System



September 2010

c/o Chesapeake Biological Laboratory
University of Maryland Center for Environmental Science
PO Box 38, Solomons, Maryland 20688, USA

Ref. No. [UMCES]CBL 10-038

Notice

The objective of this Maritime Environmental Resource Center (MERC) evaluation was to provide shipping lines, classification societies, regulators, and flag states with an independent and credible assessment of treatment performance under realistic conditions. Therefore, the ballast water treatment system was tested in accordance with the International Maritime Organization (IMO) International Convention for the Control and Management of Ships' Ballast Water and Sediment (2004), Resolution MEPC.174(58) *Guidelines for Approval of Ballast Water Management Systems (G8)* and Resolution MEPC.169(57) *Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9)*. The evaluation was conducted under specific, predetermined, agreed-upon protocols, criteria, and quality assurance procedures to assess the treatment system's performance.

MERC does not label or list technologies as acceptable or unacceptable but will present the results in an objective way that can be used to determine regulatory compliance by appropriate administrations, agencies or certification societies. Subsequent data on the technology's performance characteristics is presented to allow for comparison with the IMO Convention discharge standards, Regulation D-2, *Ballast Water Performance Standard*.

MERC and the MERC Advisory Board do not provide certification for technologies, or certify that a technology will always operate as demonstrated. Additionally, no expressed or implied guarantee is provided as to the performance of the technology, or that a technology will always operate at the levels verified. MERC does guarantee the levels verified during the evaluation under the conditions, circumstances, and operations encountered as fully independent and credible.

This report has been reviewed by members of the MERC Advisory Board and provided to Siemens and MERC funding agencies prior to public release. Mention of trade names or commercial products does not constitute endorsement or recommendation by MERC.

Questions and comments should be directed to Dr. Mario Tamburri, tamburri@umces.edu.

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1.0 MERC Background and Objectives

The Maritime Environmental Resource Center (MERC) is a State of Maryland initiative that provides test facilities, information, and decision tools to address key environmental issues facing the international maritime industry. The Center's primary focus is to evaluate the mechanical and biological efficacy, associated costs, and logistical aspects of ballast water treatment systems and the economic impacts of ballast water regulations and management approaches. A full description of MERC's structure, products, and services can be found at www.maritime-enviro.org.

To address the need for effective, safe, and reliable ballast water treatment systems to prevent the introduction of non-native species, MERC has developed as a partnership between the Maryland Port Administration (MPA), Chesapeake Biological Laboratory/ University of Maryland Center for Environmental Science (CBL/UMCES), U.S. Maritime Administration (MARAD), Smithsonian Environmental Research Center (SERC), and University of Maryland (UMD) to provide independent performance testing and to help facilitate the transition of new treatment technologies to shipboard implementation and operations.

This report describes the MERC evaluation of the Siemens SiCURE™ Ballast Water Management System through objective and quality assured land-based testing (dockside at a flow rate of 200m³/hr). The goal of this evaluation was to provide shipping lines, regulators, classification societies, and flag states with an independent and credible assessment of treatment performance under realistic conditions. Therefore, the data and information on performance characteristics covers legitimate information to meet the evaluation's objective, and performance is presented in a way to allow for comparison against the International Maritime Organization (IMO) International Convention for the Control and Management of Ships' Ballast Water and Sediments (2004), Regulation D-2 *Ballast Water Performance Standard*.

2.0 Description of the Siemens Ballast Water Management System

The Siemens Water Technologies SiCURE™ Ballast Water Management System (BWMS) utilizes a combination of treatment methods including physical separation and a proprietary, on-demand treatment with biocides produced in-situ from seawater without the addition of chemicals. This proprietary technology is based on the maritime industry-proven Chloropac® biofouling control technology that was first developed in the early 1970s and installed on over 2,500 vessels.

The SiCURE™ system employs electrolysis of seawater to produce a dilute solution of sodium hypochlorite as an Active Substance that is injected into the ballast piping. The proprietary controls regulate the system's parameters to provide only as much Active Substance as required to achieve the necessary level of disinfection. This approach is aimed at eliminating over-chlorination, associated risks of corrosion, and generation of disinfection by-products.

The specific SiCURE™ unit tested as part of this MERC land-based evaluation was a prototype designed to land-based evaluation conditions with shipboard implementation considerations. For this land based test, the water is first filtered through a 40 micron BallastSafe™ BSFc Automatic Electric Filter, Model BSFc-H-1.6 prior to treatment. The sintered stainless steel screen technology enables it to remove zooplankton. BallastSafe's filter features continuous cleaning of large volumes of dirt during ballasting without interruption, and a reversible screw system for cleaning of the entire screen surface. This system requires the

discharge/backflush of accumulated solid residue (or retentate) overboard or into a separate tank during filling operations.

To account for brackish water conditions, the addition of a brine (sodium chloride) injection system was included for proper operation during periods of relatively low salinity. The sodium hypochlorite solution was produced from a small side stream of ballast water from the main ballast piping. An advanced electrolyzer treated the side stream to create the required Active Substance concentration on-demand. The sodium hypochlorite/Active Substance solution was then injected both before and after the filtration unit. The pre filtration injection accounted for 10% or less of the total treatment dose for the purpose of filter biofouling prevention. An injection quill specifically designed for this application was implemented for the primary dose after the filter unit. The SiCURE™ system doses at a maximum of 6 mg/l total chlorine. Upon discharge of treated water, total chlorine levels are monitored and if the levels are greater than the discharge limit of 0.1 ppm, an automated system injects sodium bisulfite as neutralizer.

Because the treatment system was a prototype, it was operated at all times by members of the Siemens staff. All evaluation test equipment and instrumentation was operated by MERC personnel.

3.0 Summary of IMO Standards

This evaluation was designed to determine if the SiCURE™ treatment system could meet IMO D2 standards in accordance with both the IMO *Guidelines for Approval of Ballast Water Management Systems (G8)* and the *Procedure for Approval of Ballast Water Management Systems that make use of Active Substances (G9)*. The IMO Convention performance standard states that ships must discharge:

- 1) Less than 10 viable organisms per m³, greater than or equal to 50 µm in minimum dimension;
- 2) Less than 10 viable organisms per ml, less than 50 µm in minimum dimension and greater than or equal to 10 µm in minimum dimension and
- 3) Less than the following concentrations of indicator microbes, as a human health standard:
 1. Toxigenic *Vibrio cholerae* (serogroups O1 and O139), less than 1 colony forming unit (cfu) per 100 ml
 2. *Escherichia coli*, less than 250 cfu per 100 ml;
 3. Intestinal *Enterococci*, less than 100 cfu per 100 ml.

4.0 Summary of Test Protocols

The following is a brief summary of the testing approach and methods. For complete details on protocols, data management, and quality control / quality assurance procedures for this MERC evaluation, please refer to the *Test Plan for the Performance Evaluation of the Siemens Ballast Water Management System* (August 2009), available for download at www.maritime-enviro.org.

The protocols described below are based upon the IMO G8/G9 guidelines and the U.S. Coast Guard supported ETV protocols under development. Any deviation from IMO G8/G9 guidelines or draft ETV protocols were explained and justified in the Test Plan.

MERC evaluated the biological efficacy of the SiCURE™ ballast water treatment system onboard the U.S. Maritime Administration (MARAD) Ro-Ro vessel *MV Cape Washington* while

docked in the Port of Baltimore. The ballast system on *MV Cape Washington* was modified to allow for water at a flow rate of 400m³/hr to be split equally at flow rates of 200 m³/hr. Just before this split, challenge condition concentrations of total suspended solids (TSS) and particulate carbon (POC) were augmented by injecting a concentrated slurry of Arizona test dust and humic acid (developed and validated by the Naval Research Laboratory, Key West, Florida). The water was then delivered simultaneously to either a “control” (untreated) ballast tank or a “treated” (passing first through the SiCURE™ system) ballast tank. These two tanks were used for the required holding time of five days and were essentially identical in size and structure. Each tank was filled to approximately 250 m³ for each test trial.

Physical Parameters - Temperature, salinity, dissolved oxygen, chlorophyll fluorescence, turbidity and pH were measured every 15 minutes during the test trials by two identical multi-parameter probes placed, one each, into the control and test tanks. Initial inline samples of ballast water during the filling of the control and test tanks were collected, filtered, and analyzed (using USEPA methods) for the water quality parameters of particulate organic carbon (POC), dissolved organic carbon (DOC) and total suspended solids (TSS) by the CBL/UMCES Nutrient Analytical Services Laboratory (NASL).

Sampling - A total of 10 identical 1.1 m³ conical bottom mesocosms were also used for controlled sampling during each trial. Using the mesocosms, five sequential samples were taken during: (A) initial filling of tanks, just prior to the split of control and treated water (T0 Control), (B) initial filling of test tank, just downstream of the SiCURE™ system during filling of test tank (T0 Treated), (C) during discharge of control water after a five-day holding time (TF Control), and (D) during discharge of treated water after a five-day holding time (TF Treated).

Live Organisms > 50 µm - Immediately after filling, 1.0 m³ of water in each mesocosm was filtered through a 35 µm plankton net to concentrate the zooplankton for qualitative and quantitative analyses under a dissecting microscope. The proportion and total concentration of live versus dead organisms was determined using standard movement and response-to-stimuli techniques within one hour of collecting the individual samples. Zooplankton samples were also fixed and returned to the laboratory for additional taxonomic evaluations.

Live Organisms 10 - 50 µm - Fifteen to twenty liters of well-mixed, but unfiltered water from each mesocosm were also collected immediately after filling, to determine concentrations of organisms in the 10 to 50 micron size class using three distinct methods: (A) A sub-sample was stained using a combination of CMFDA (5-chloromethylfluorescein diacetate) and FDA (fluorescein diacetate) as selective live/viable indicators. Stained sub-samples were incubated and observed on a Sedgewick Rafter slide using a Leitz Laborlux S modified for epifluorescence. (B) A second sub-sample was fixed with standard Lugol's solution for use in taxonomic identifications and to serve as a backup sample. (C) A third sub-sample was filtered and frozen until analysis of total and active chlorophyll-a by the NASL. (D) Finally, a fourth sub-sample was used to determine chlorophyll-a levels after allowed to regrow under favorable conditions. An increase in chlorophyll, or positive regrowth, indicates that viable phytoplankton were in the samples, whereas chlorophyll levels at or below detection limits of the laboratory analytical method suggests that there was no viable phytoplankton.

Live Microbes - Additional subsamples of unfiltered water were also collected from each mesocosm to determine concentrations of total heterotrophic bacteria and three specific indicator pathogens, *E. coli*, intestinal *Enterococci*, and toxigenic *Vibrio cholerae*. Total heterotrophic bacteria were enumerated by spread plate method using NWRI agar. The presence and abundance of intestinal *Enterococci* was determined using a commercially available

chromogenic substrate method. Culturable *E. coli* concentrations were determined using a standard USEPA method: membrane filtration on modified mTEC agar. Abundances of total and toxigenic *V. cholerae* were calculated by filtration and selection on TCBS agar and enumerated using a species-specific RNA colony blot and *ctxA* DNA colony blot hybridization. Viable toxigenic *V. cholerae* was assayed with a commercial DFA kit specific for serogroup O1 using monoclonal antibodies tagged with fluorescein isothiocyanate.

Toxicity - To evaluate the toxicity of treated water at the completion of each trial, samples from each mesocosm were collected and tested for chronic toxicity and for total residual chlorine. Filter “backflush” (retenate/filtrate during initial treatment of water) was also tested for total residual chlorine. The toxicity protocols and species used were consistent with the USEPA methods for Whole Effluent Toxicity (WET). The algal species tested was *Isochrysis galbana*, the fish species was the Sheepshead minnow (*Cyprinodon variegatus*) while the invertebrate species was the Mysid shrimp (*Americamysis bahia*).

Chlorine concentration in samples was analyzed immediately upon collection to avoid potential loss of oxidant with time. The *Standard Methods for the Examination of Water and Wastewater Low-Level Amperometric Titration* method 4500-Cl D and DPD Colorimetric method 4500-Cl G were used to measure Total Residual Oxidants (TRO). A Fischer and Porter amperometric titrator was also used for amperometric measurements.

To assess disinfection by-products, a nine-liter carboy of water was drawn at the following 4 time-points: T-0 Control inflow, T-0 Treated inflow, T-0 backflush discharge and T-F Treated discharge. The carboy of water was immediately sampled for analyses of the following compounds: Trihalomethanes (TTHM), Haloacetic Acids (HAA5), Tribromoacetic Acid (TBAA), Bromate (Br), Chlorate (ClO₃), Sodium (Na, as a metal), and various Nitriles (-CN group – triple bond). Samples were iced and delivered same-day via courier to the Analytical Laboratory Services, Inc. (ALSI) for analysis.

5.0 Summary of Results

Live Organisms - For essentially all biological categories, the Siemens SiCURE™ BWMS reduced the numbers of live organisms in ballast water to levels below IMO D2 discharge standards. The only anomaly was found in one trial where *Enterococci* abundances increased slightly in both treated and control water during the 5-day hold time, with a TF value just over (107.4 ± 33.5) the IMO D2 discharge standard of less than 100 cfu/100 ml. However, it is important to note the relatively large standard deviation, that the most probable number (MPN) method used (Idexx Enterolert kit) to quantify *Enterococci* commonly over estimate abundance when compared to the a traditional membrane filtration method (G. Ditcher, personal communication), and in all other test trials (over two different years) *Enterococci* appeared to be successfully treated by the SiCURE™ system.

Chronic Toxicity - Toxicity testing was conducted on five treated discharge samples between 4/27/10 and 6/15/10. Results indicate that treated ballast water discharge (TF) was not chronically toxic to mysid shrimp or Sheepshead Minnows. However the treated water upon discharge did significantly inhibit the growth of the algae species, *Isochrysis galbana* (T-Iso.) for three of the test trials (Trial 1 22-27 April, Trial 2 13-18 May, and Trial 5 3-8 June), which is reported as “toxic” for this algal assay. These samples had LOECs of 100% and NOECs of 56%.

All three algae tests with a toxicity response in the undiluted sample (100%) had similar EC50s of 70%, 71% and 78% for SIE-03, -05 and -08, respectively.

Residual Chlorine Analysis - Levels of residual chlorine (or Total Residual Oxidants - TRO) were below the permissible discharge limit of 0.10 ppm in all TF treated samples. Values ranged from 0.06 ppm to below the method detection limit (MDL) of 0.02 ppm. A summary table from the completed trial runs and the 24-hour dechlorination run is provided below. TRO was immediately measured shipboard using the DPD colorimetric analysis method.

Note that Total Residual Chlorine (TRC) is not directly measured using the DPD colorimetric analysis method. The analysis does not distinguish between various oxidants (chlorine, bromine, ozone, etc). It reports TRO in chlorine equivalents (in other words, as if all oxidant measured were chlorine). In freshwater, after introducing chlorine, we would be comfortable calling the residual TRC. However, it is likely that in saltwater, while the majority are chlorines, some portion may well be bromines. Thus, we only report TRO.

TRO as measured in the control and treated tanks.

Report ID	Run ID	Date	TRO Treated Tank	TRO Control Tank	Comment
Trial 1	SIE-03-10	4/27	0.06 ppm		
Trial 2	SIE-05-10	5/18	0.08 ppm	0.01 ppm	
Trial 3	SIE-06-10	5/25	0.09 ppm	0.03 ppm	
Trial 4	SIE-07-10	6/1	ND	ND	
Trial 5	SIE-08-10	6/8	0.06 ppm	0.04 ppm	
24-Hour Trial	SIE-09-10	6/10	0.59 ppm	0.06 ppm	Initial
			0.20 ppm		Thiosulfate
			0.21 ppm		Thiosulfate
			0.22 ppm		Thiosulfate
			0.04 ppm		Bisulfite
			0.04 ppm		Bisulfite
			0.02 ppm		Bisulfite
			0.03 ppm		Bisulfite
			0.06 ppm		Bisulfite
			0.03 ppm		Bisulfite

Notes from dechlorination run.

1. Thiosulfate treatment did not result in adequate dechlorination at point of discharge.
2. Measured TRO levels in bisulfite treated samples were consistently below measured free (active) chlorine levels, suggesting interference of bisulfite with DPD colorimetric analysis.

Disinfection By-Products - A summaries of the results for DBP analyses for each trial are in the results section below. Reporting detection limits are provided.

Twenty four-hour dechlorination trial - On June 9-10, 2010, MERC conducted one additional trial that required the use of the Siemens dechlorination system. While TF water in all 5-day hold time trials had total chlorine levels below 0.1 ppm, a short 24-hour hold time resulted in measurable levels of chlorine and the subsequent automate dechlorination of the treated water upon discharge using sodium bisulfate. For this added trial, total chlorine levels in discharged treated water declined in from 0.59 ppm prior to dechlorination, to below 0.1 ppm after the

addition sodium bisulfate. Note an attempt at using sodium thiosulfate on discharge was not effective, resulting in a final chlorine level of 0.21 ppm.

Mechanical Failures - SIE-10-02-T0 (15 April 2010) The filtration unit on the SiCURE™ system failed prior to the first sampling mesocosm being completely filled. The unit clogged, causing a complete stoppage of flow. Trial SIE-10-02-T0 was therefore canceled. SIE-10-06-T0 (20 May 2010) The SiCURE™ system experienced a malfunction during startup but prior to the filling of the treated tank and sampling mesocosms. Once the issue was resolved, testing was re-initiated. It is important to note that, in all trials, Siemens required 10-15 minutes of flow through their system before electrochlorination was initiated. This flow was diverted into the a separated ballast tanks and not into the treated test tank.

* Complete datasets and further performance information is available upon request.

6.0 Results

6.1. Trial 1 (SIE-03-10): 22-27 April 2010

Presented as means and standard deviations.

Physical Parameters

	TSS mg/l	DOC mg/l	PC mg/l
T0 Ambient	23.9 (3.7)	2.82 (0.01)	3.44 (0.03)
T0 Enhanced	51.3 (2.4)	NA	5.61 (0.61)

Enhanced data is the average of 3 time points (2 reps per time point) during the fill time of the ballast tanks.

Ambient water is sampled at the beginning of the trial and before the water is enhanced.

	Temp °C	Salinity psu	Dis. Oxygen mg/l	Turbidity NTU	pH
T-0 Control	14.28 (0.03)	5.97 (0.00)	11.89 (0.03)	17.39 (1.10)	8.5 (0.1)
T-0 Treated	14.36 (0.02)	6.64 (0.00)	16.24 (0.09)	18.46 (1.21)	8.6 (0.1)
T-F Control	14.14 (0.01)	5.95 (0.01)	8.37 (0.02)	3.67 (0.19)	7.8 (0.1)
T-F Treated	14.18 (0.01)	6.61 (0.01)	10.93 (0.01)	6.56 (0.09)	8.0 (0.1)

Live Organisms > 50 µm

	T0 #/m ³	TF #/m ³
Control	124,000 (5,000)	*223,667 (29,501)
Treated	4.5 (7)	5 (4.5)

*Note large increase between T0 and TF in Control tank (reproduction and hatching of eggs).

*TF Control	*TF Treated
Copepod nauplii Calanoida (<i>Acartia sp.</i>) Cirripedia nauplii Harpacticoida	Copepoda Rotifera

* Up to four top taxa listed in order of abundance.

Live Organisms 10 - 50 μ m

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up these stains.

	T0 #/ml	TF #/ml
Control	5,297 (776)	1,113 (225)
Treated	29 (19)	0 (0)

Dominant species	Type	Other
<i>Heterocapsa rotundatum</i>	Dinoflagellate	Small but abundant
<i>Gymnodinium sp.</i>	Dinoflagellate	
<i>Navicula sp.</i>	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms.

	T0 μg/l	TF μg/l	RG μg/l
Control	39.84 (11.77)	5.63 (0.50)	1.19 (0.13)
Treated	0.32 (0.13)	0.21 (0.02)	0.00 (0.01)

RG = after re-growth assay; MDL = 0.56 μ g/l

Live Microbes

cfu = colony forming units

<i>E. coli</i>	T0 cfu/100ml	TF cfu/100ml
Control	3.4 (1.52)	0.6 (1.34)
Treated	0 (0)	0 (0)

<i>Enterococci</i>	T0 NPM/100ml	TF MPN/100ml
Control	3.92 (1.38)	9.06 (2.96)
Treated	1 (0.71)	1.2 (0.45)

MPN = most probably number estimate determined by the Idexx Enterolert kit.

V. cholerae – No detectable culturable toxigenic *Vibrio cholerae* were detected in any samples during any of the trials.

<i>Heterotrophic Bacteria</i>	T0 (sd) cfu/1ml	TF (sd) cfu/1ml
Control	1,518 (373.6)	808 (190.66)
Treated	2.2 (3.58)	29,120 (2,184.49)

Toxicity Summaries

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were below the permissible discharge limit of 0.10 ppm in all TF treated samples. Values ranged from 0.06 ppm to below the method detection limit (MDL) of 0.02 ppm.

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows. All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls.

Disinfection By-Products (µg/l)

	Control	Treated	BW	Treated	RDL/MRL
	T0	T0	T0	TF	
Trihalomethanes	0	91.2	ND	293	0.5
Trichloromethane	< 0.5	< 0.5	ND	< 0.5	0.5
Bromodichloromethane	< 0.5	2.7	ND	3.8	0.5
Dibromochloromethane	< 0.5	20.7	ND	28.2	0.5
Tribromomethane	< 0.5	67.8	ND	261	0.5
Haloacetic Acids	0	87.4	ND	197	1.0
Monochloroacetic acid	< 2.0	< 2.0	ND	< 2.0	2.0
Dichloroacetic acid	< 1.0	1.2	ND	< 1.0	1.0
Trichloroacetic acid	< 1.0	< 1.0	ND	< 1.0	1.0
Bromochloroacetic acid	< 1.0	5	ND	< 1.0	1.0
Monobromoacetic acid	< 1.0	4.5	ND	< 1.0	1.0
Dibromoacetic acid	< 1.0	72.2	ND	< 1.0	1.0
Tribromoacetic acid	< 1.0	4.5	ND	197	1.0
Other			ND		
Sodium chlorate	< 10	340	ND	340	10
Sodium bromate	< 50.0	< 50.0	ND	< 50.0	50.0
Monochloroacetonitrile	<0.5	<0.5	ND	<0.5	
Dichloroacetonitrile	<0.5	<0.5	ND	<0.5	0.5
Monobromoacetonitrile	<0.5	<0.5	ND	<0.5	
Dibromoacetonitrile	<0.5	18	ND	<0.5	0.5
Sodium	1710	1910	ND	1870	1.5
NaCl (calculated)	4349	4858	ND	4756	

BW = backwash; RDL = reporting detection limit; MRL = method reporting limit

6.2. Trial 2 (SIE-05-10): 13-18 May 2010

Presented as means and standard deviations.

Physical Parameters

	TSS mg/l	DOC mg/l	POC mg/l
T0 Ambient	11.5 (1.0)	3.41 (0.13)	1.11 (0.03)
T0 Enhanced	65.2 (8.5)	NA	5.88 (0.79)

Enhanced data is the average of 3 time points (2 reps per time point) during the fill time of the ballast tanks.

Ambient water is sampled at the beginning of the trial and before the water is enhanced.

	Temp °C	Salinity psu	Dis. Oxygen mg/l	Turbidity NTU	pH
T-0 Control	15.15 (0.01)	8.19 (0.02)	8.54 (0.06)	15.60 (0.87)	7.3 (0.1)
T-0 Treated	15.20 (0.01)	8.84 (0.01)	9.02 (0.02)	15.11 (1.24)	7.3 (0.1)
T-F Control	16.53 (0.06)	8.15 (0.00)	6.92 (0.06)	3.49 (0.23)	7.2 (0.2)
T-F Treated	16.52 (0.05)	8.76 (0.00)	8.74 (0.03)	5.27 (0.42)	7.1 (0.1)

Live Organisms > 50 µm

	T0 #/m ³	*TF #/m ³
Control	60,000 (10,500)	90,500 (10,000) †
Treated	4.2 (7)	0.2 (0.4)

*Note moderate increase between T0 and TF in Control tank (reproduction and hatching of eggs).

*TF Control	*TF Treated
Copepod nauplii Calanoida (<i>Acartia sp.</i>) Polychaeta (Spionidae) Harpacticoida	Polycheata (Spionidae)

* Up to four top taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up these stains.

	T0 #/ml	TF #/ml
Control	1,915 (511)	3,182 (725)
Treatment	1.7 (1.2)	6 (3.8)

Dominant species	Type	Other
<i>Heterocapsa rotundatum</i>	Dinoflagellate	chain-forming
<i>Gymnodinium sp.</i>	Dinoflagellate	
<i>Thalassiosira gravida</i>	Diatom	
<i>Navicula sp.</i>	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms.

	T0 µg/l	TF µg/l	RG µg/l
Control	8.79 (0.48)	1.89 (0.06)	5.95 (3.11)
Treated	0.08 (0.01)	0.03 (0.01)	0.08 (0.09)

RG = after re-growth assay

MDL = 0.56 µg/l

Live Microbes

cfu = colony forming units

<i>E. coli</i>	T0 cfu/100ml	TF cfu/100ml
Control	4.6 (1.82)	0 (0)
Treated	0 (0)	1 (1.41)

<i>Enterococci</i>	T0 MPN/100ml	TF MPN/100ml
Control	8.78 (21.2)	49.34 (26.54)
Treated	2.02 (1.45)	107.4 (33.5)

MPN = most probably number estimate determined by the Idexx Enterolert kit. Note that this MPN is not a direct cfu count and Enterolert MPN values are commonly found to be higher than the traditional membrane filtration method used to estimate *Enterococci* abundances (G. Ditcher, Idexx).

V. cholerae – No detectable culturable toxigenic *Vibrio cholerae* were detected in any samples during any of the trials.

<i>Heterotrophic Bacteria</i>	T0 cfu/1ml	TF cfu/1ml
Control	146 (80)	503.33 (187.75)
Treated	0 (0)	10,520 (4,447.13)

Toxicity Summaries

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were below the permissible discharge limit of 0.10 ppm in all TF treated samples. Values ranged from 0.06 ppm to below the method detection limit (MDL) of 0.02 ppm.

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows. All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls.

Disinfection By-Products (µg/l)

	Control	Treated	BW	Treated	RDL/MRL
	T0	T0	T0	TF	
Trihalomethanes	0	106.6	ND	227.8	0.5
Trichloromethane	< 0.5	< 0.5	ND	< 0.5	0.5
Bromodichloromethane	< 0.5	1.6	ND	2.5	0.5
Dibromochloromethane	< 0.5	13.5	ND	24.3	0.5
Tribromomethane	< 0.5	91.5	ND	201	0.5
Haloacetic Acids	1.4	62.9	ND	385.6	1.0
Monochloroacetic acid	< 2.0	< 2.0	ND	< 2.0	2.0
Dichloroacetic acid	< 1.0	< 1.0	ND	< 1.0	1.0
Trichloroacetic acid	< 1.0	< 1.0	ND	< 1.0	1.0
Bromochloroacetic acid	< 1.0	3.3	ND	< 1.0	1.0
Monobromoacetic acid	< 1.0	3.1	ND	1	1.0
Dibromoacetic acid	< 1.0	53	ND	10.6	1.0
Tribromoacetic acid	1.4	3.5	ND	374	1.0
Other			ND		
Sodium chlorate	< 10	250	ND	250	10
Sodium bromate	< 50.0	< 50.0	ND	< 50.0	50.0
Monochloroacetonitrile	<0.5	<0.5	ND	<0.5	
Dichloroacetonitrile	<0.5	0.86	ND	<0.5	0.5
Monobromoacetonitrile	<0.5	<0.5	ND	<0.5	
Dibromoacetonitrile	<0.5	24	ND	4.4	0.5
Sodium	2330	2670	2400??	2790	1.5
NaCl (calculated)	5926	6791	6104??	7096	

BW = backwash

RDL = reporting detection limit

MRL = method reporting limit

6.3. Trial 3 (SIE-06-10): 20-25 May 2010

Presented as means and standard deviations.

Physical Parameters

	TSS mg/l	DOC mg/l	POC mg/l
T0 Ambient	19.7 (3.6)	2.96 (0.00)	1.28 (0.01)
T0 Enhanced	56.2 (13.5)	NA	4.76 (0.92)

Enhanced data is the average of 3 time points (2 reps per time point) during the fill time of the ballast tanks.

Ambient water is sampled at the beginning of the trial and before the water is enhanced.

	Temp °C	Salinity psu	Dis. Oxygen mg/l	Turbidity NTU	pH
T-0 Control	15.14 (0.02)	9.80 (0.02)	6.47 (0.39)	15.68 (1.30)	7.1 (0.1)
T-0 Treated	15.18 (0.00)	10.35 (0.00)	7.91 (0.01)	14.95 (1.01)	7.0 (0.1)
T-F Control	19.83 (0.01)	9.75 (0.00)	4.82 (0.02)	3.06 (0.10)	7.1 (0.1)
T-F Treated	19.97 (0.01)	10.37 (0.00)	7.55 (0.01)	6.61 (0.09)	7.0 (0.1)

Live Organisms > 50 µm

	T0 #/m³	TF #/m³
Control	50,500 (10,000)	35,666 (2,350)
Treated	9 (7)	9 (3.5)

*TF Control	*TF Treated
Copepod nauplii	Bivalve larvae
Polychaeta (Spionidae)	Copepoda
Calanoida (<i>Acartia sp.</i> , <i>Eurytemora sp.</i>)	Turbellaria
Harpacticoida	

* Up to four top taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up these stains.

	T0 #/ml	TF #/ml
Control	1,088 (194)	137 (8)
Treatment	1.7 (1.2)	0 (0)

Dominant species	Type	Other
<i>Heterocapsa rotundatum</i>	Dinoflagellate	chain-forming
<i>Gymnodinium sp.</i>	Dinoflagellate	
<i>Thalassiosira gravida</i>	Diatom	
<i>Navicula sp.</i>	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms.

	T0 µg/l	TF µg/l	RG µg/l
Control	3.07 (0.09)	0.91 (0.02)	0.12 (0.09)
Treated	0.24 (0.17)	0.13 (0.02)	0.00 (0.00)

RG = after re-growth assay; MDL = 0.56 µg/l

Live Microbes

cfu = colony forming units

<i>E. coli</i>	T0 cfu/100ml	TF cfu/100ml
Control	0 (0)	2.2 (1.5)
Treated	0 (0)	0 (0)

<i>Enterococci</i>	T0 MPN/100ml	TF MPN/100ml
Control	16.64 (4.08)	70.42 (29.77)
Treated	5.8 (1.9)	34.68 (8.59)

MPN = most probably number estimate determined by the Idexx Enterolert kit.

V. cholerae – No detectable culturable toxigenic *Vibrio cholerae* were detected in any samples during any of the trials.

<i>Heterotrophic Bacteria</i>	T0 cfu/1ml	TF cfu/1ml
Control	950 (300)	55 (31)
Treated	0 (0)	13,780 (2,560)

Toxicity Summaries

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were below the permissible discharge limit of 0.10 ppm in all TF treated samples. Values ranged from 0.06 ppm to below the method detection limit (MDL) of 0.02 ppm.

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to any of the three tested species.

Disinfection By-Products (µg/l)

	Control	Treated	BW	Treated	RDL/MRL
	T0	T0	T0	TF	
Trihalomethanes	0	27	0.63	168.2	0.5
Trichloromethane	< 0.5	< 0.5	< 0.5	< 0.5	0.5
Bromodichloromethane	< 0.5	1.2	< 0.5	2.3	0.5
Dibromochloromethane	< 0.5	10.6	< 0.5	17.9	0.5
Tribromomethane	< 0.5	15.2	0.63	148	0.5
Haloacetic Acids	0	71.1	1.9	6.9	1.0
Monochloroacetic acid	< 2.0	< 2.0	< 2.0	< 2.0	2.0
Dichloroacetic acid	< 1.0	< 1.0	< 1.0	< 1.0	1.0
Trichloroacetic acid	< 1.0	< 1.0	< 1.0	1.4	1.0
Bromochloroacetic acid	< 1.0	2.9	< 1.0	< 1.0	1.0
Monobromoacetic acid	< 1.0	1.4	< 1.0	< 1.0	1.0
Dibromoacetic acid	< 1.0	28.4	< 1.0	5.5	1.0
Tribromoacetic acid	< 4.0	38.4	1.9	0	1.0
Other					
Sodium chlorate	< 10	170	< 10	< 10	10
Sodium bromate	< 50.0	< 50.0	< 50.0	< 50.0	50.0
Monochloroacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dichloroacetonitrile	<0.5	<0.5	<0.5	<0.5	0.5
Monobromoacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dibromoacetonitrile	<0.5	15	<0.5	7.4	0.5
Sodium	2830	3060	2920	3170	1.5
NaCl (calculated)	7198	7783	7427	8063	

BW = backwash

RDL = reporting detection limit

MRL = method reporting limit

6.4. Trial 4 (SIE-07-10): 27 May – 1 June 2010

Presented as means and standard deviations.

Physical Parameters

	TSS mg/l	DOC mg/l	POC mg/l
T0 Ambient	12.7 (0.4)	3.16 (0.02)	1.29 (0.04)
T0 Enhanced	57.9 (1.7)	NA	5.36 (0.19)

Enhanced data is the average of 3 time points (2 reps per time point) during the fill time of the ballast tanks.

Ambient water is sampled at the beginning of the trial and before the water is enhanced.

	Temp °C	Salinity psu	Dis. Oxygen mg/l	Turbidity NTU	pH
T-0 Control	17.40 (0.03)	8.81 (0.03)	7.09 (0.10)	15.84 (1.11)	6.9 (0.1)
T-0 Treated	17.48 (0.02)	9.42 (0.00)	5.47 (0.04)	16.09 (1.21)	7.0 (0.1)
T-F Control	19.53 (0.01)	8.87 (0.00)	5.18 (0.10)	2.22 (0.08)	7.2 (0.1)
T-F Treated	19.69 (0.01)	9.48 (0.00)	5.57 (0.02)	6.07 (0.13)	7.2 (0.1)

Live Organisms > 50 µm

	T0 #/m ³	TF #/m ³
Control	113,333 (14,154)	63,500 (9,750)
Treated	13 (12)	1.8 (0.8)

*TF Control	*TF Treated
Calanoida (<i>Eurytemora</i> sp.)	Copepoda
Copepod nauplii	Bivalve larva
Polychaeta (Spionidae)	
Bivalve larvae	

* Up to four top taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up these stains.

	T0 #/ml	TF #/ml
Control	1,544 (103)	1,026 (133)
Treatment	1 (1)	1.2 (0.8)

Dominant species	Type	Other
<i>Prorocentrum minimum</i>	Dinoflagellate	first time detected in 2010 chain-forming
<i>Heterocapsa rotundatum</i>	Dinoflagellate	
<i>Gymnodinium estuarale</i>	Dinoflagellate	
<i>Thalassiosira</i> sp.	Diatom	
<i>Navicula</i> sp.	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms.

	T0 µg/l	TF µg/l	RG µg/l
Control	6.42 (0.78)	1.08 (0.04)	1.41 (0.47)
Treated	0.05 (0.01)	0.02 (0.01)	0.00 (0.00)

RG = after re-growth assay

MDL = 0.56 µg/l

Live Microbes

cfu = colony forming units

<i>E. coli</i>	T0 cfu/100ml	TF cfu/100ml
Control	2.2 (2.49)	0 (0)
Treated	0 (0)	0 (0)

<i>Enterococci</i>	T0 MPN/100ml	TF MPN/100ml
Control	10 (4)	11.62 (3.75)
Treated	2.46 (2.16)	57.96 (30.54)

MPN = most probably number estimate determined by the Idexx Enterolert kit.

V. cholerae – No detectable culturable toxigenic *Vibrio cholerae* were detected in any samples during any of the trials.

<i>Heterotrophic Bacteria</i>	T0 cfu/1ml	TF cfu/1ml
Control	470 (176)	190 (63.07)
Treated	1.4 (1.43)	7,630 (4,181)

Toxicity Summaries

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were below the permissible discharge limit of 0.10 ppm in all TF treated samples. Values ranged from 0.06 ppm to below the method detection limit (MDL) of 0.02 ppm.

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to any of the three tested species.

Disinfection By-Products (µg/l)

	Control	Treated	BW	Treated	RDL/MRL
	T0	T0	T0	TF	
Trihalomethanes	0	98.2	0	281.2	0.5
Trichloromethane	< 0.5	< 0.5	< 0.5	< 0.5	0.5
Bromodichloromethane	< 0.5	1.6	< 0.5	2.6	0.5
Dibromochloromethane	< 0.5	14.9	< 0.5	25.6	0.5
Tribromomethane	< 0.5	81.7	< 0.5	253	0.5
Haloacetic Acids	0	56.7	0	117.6	1.0
Monochloroacetic acid	< 2.0	< 2.0	< 2.0	< 2.0	2.0
Dichloroacetic acid	< 1.0	< 1.0	< 1.0	< 1.0	1.0
Trichloroacetic acid	< 1.0	< 1.0	< 1.0	< 1.0	1.0
Bromochloroacetic acid	< 1.0	4.1	< 1.0	< 1.0	1.0
Monobromoacetic acid	< 1.0	3.4	< 1.0	< 1.0	1.0
Dibromoacetic acid	< 1.0	49.2	< 1.0	10.6	1.0
Tribromoacetic acid	< 4.0	0	< 4.0	107	1.0
Other					
Sodium chlorate	< 10	180	< 10	240/200	10
Sodium bromate	< 50.0	< 50.0	< 50.0	< 50.0	50.0
Monochloroacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dichloroacetonitrile	<0.5	<0.5	<0.5	<0.5	0.5
Monobromoacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dibromoacetonitrile	<0.5	26	<0.5	12	0.5
Sodium	2570	2940	2610	N/A	1.5
NaCl (calculated)	6537	7478	6638	#VALUE!	

BW = backwash

RDL = reporting detection limit

MRL = method reporting limit

6.5. Trial 5 (SIE-08-10): 3-8 June 2010

Presented as means and standard deviations.

Physical Parameters

	TSS mg/l	DOC mg/l	POC mg/l
T0 Ambient	17.1 (5.1)	3.76 (0.11)	1.56 (0.18)
T0 Enhanced	55.0 (5.6)	NA	5.39 (0.52)

Enhanced data is the average of 3 time points (2 reps per time point) during the fill time of the ballast tanks.

Ambient water is sampled at the beginning of the trial and before the water is enhanced.

	Temp °C	Salinity psu	Dis. Oxygen mg/l	Turbidity NTU	pH
T-0 Control	20.48 (0.02)	7.41 (0.00)	4.82 (0.15)	15.96 (1.13)	7.1 (0.1)
T-0 Treated	20.52 (0.01)	6.99 (0.01)	7.89 (0.01)	16.05 (1.14)	7.1 (0.1)
T-F Control	20.13 (0.02)	7.50 (0.00)	3.13 (0.04)	1.32 (0.07)	7.0 (0.1)
T-F Treated	20.27 (0.03)	6.34 (0.01)	7.94 (0.00)	7.22 (0.18)	7.0 (0.1)

Live Organisms > 50 µm

	T0 #/m ³	TF #/m ³
Control	175,000 (11,000)	94,500 (8,250)
Treated	221 (59)	8 (3)

*TF Control	*TF Treated
Copepod nauplii	Copepoda
Bivalve larvae	Polychaeta (Spionidae)
Spionidae	
Calanoida (<i>Eurytemora sp.</i>)	

* Up to four top taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up these stains.

	T0 #/ml	TF #/ml
Control	1,511 (271)	757 (145)
Treatment	1.7 (1.2)	2.2 (1.5)

Dominant species	Type	Other
<i>Prorocentrum minimum</i>	Dinoflagellate	chain-forming
<i>Thalassiosira sp.</i>	Diatom	
<i>Gyrodinium estuarale</i>	Dinoflagellate	
<i>Navicula sp.</i>	Diatom	
<i>Amphora sp.</i>	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms.

	T0 µg/l	TF µg/l	RG µg/l
Control	3.42 (0.12)	0.44 (0.14)	0.97 (0.36)
Treated	0.04 (0.01)	0.01 (0.00)	0.00 (0.00)

RG = after re-growth assay

MDL = 0.56 µg/l

Live Microbes

cfu = colony forming units

<i>E. coli</i>	T0 cfu/100ml	TF cfu/100ml
Control	13.4 (5.5)	0 (0)
Treated	0 (0)	0 (0)

<i>Enterococci</i>	T0 MPN/100ml	TF MPN/100ml
Control	113.94 (38.9)	2.42 (0.58)
Treated	1.02 (1.45)	1 (1)

MPN = most probably number estimate determined by the Idexx Enterolert kit.

V. cholerae – No detectable culturable toxigenic *Vibrio cholerae* were detected in any samples during any of the trials.

<i>Heterotrophic Bacteria</i>	T0 cfu/1ml	TF cfu/1ml
Control	577 (149)	466 (671)
Treated	2.3 (2.5)	3,950 (884)

Toxicity Summaries

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were below the permissible discharge limit of 0.10 ppm in all TF treated samples. Values ranged from 0.06 ppm to below the method detection limit (MDL) of 0.02 ppm.

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows.

All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls.

Disinfection By-Products (µg/l)

	Control	Treated	BW	Treated	RDL/MRL
	T0	T0	T0	TF	
Trihalomethanes	0	241.4	0	89.6	0.5
Trichloromethane	< 0.5	< 0.5	< 0.5	< 0.5	0.5
Bromodichloromethane	< 0.5	2.6	< 0.5	3.3	0.5
Dibromochloromethane	< 0.5	119.8	< 0.5	24.5	0.5
Tribromomethane	< 0.5	119	< 0.5	61.8	0.5
Haloacetic Acids	0	143.8	0	95.6	1.0
Monochloroacetic acid	< 2.0	< 2.0	< 2.0	< 2.0	2.0
Dichloroacetic acid	< 1.0	< 1.0	< 1.0	< 1.0	1.0
Trichloroacetic acid	< 1.0	< 1.0	< 1.0	< 1.0	1.0
Bromochloroacetic acid	< 1.0	3.5	< 1.0	< 1.0	1.0
Monobromoacetic acid	< 1.0	3.2	< 1.0	< 1.0	1.0
Dibromoacetic acid	< 1.0	37.1	< 1.0	8.9	1.0
Tribromoacetic acid	< 4.0	100	< 4.0	86.7	1.0
Other					
Sodium chlorate	< 10	295/240	< 10	< 10/297	10
Sodium bromate	< 50.0	< 50.0	< 50.0	< 50.0	50.0
Monochloroacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dichloroacetonitrile	<0.5	<0.5	<0.5	<0.5	0.5
Monobromoacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dibromoacetonitrile	<0.5	24	<0.5	8.5	0.5
Sodium	2220	2360	2140	2700	1.5
NaCl (calculated)	5647	6003	5443	6867	

BW = backwash

RDL = reporting detection limit

MRL = method reporting limit

6.6. Twenty four-hour Dechlorination Trial: 9-10 June 2010

Presented as means and standard deviations.

Physical Parameters

	TSS mg/l	DOC mg/l	POC mg/l
T0 Ambient	33.4 (9.6)	3.11 (0.12)	2.06 (0.09)
T0 Enhanced	NA	NA	NA

Enhanced data is the average of 3 time points (2 reps per time point) during the fill time of the ballast tanks.

Ambient water is sampled at the beginning of the trial and before the water is enhanced.

	Temp °C	Salinity psu	Dis. Oxygen mg/l	Turbidity NTU	pH
T-0 Control	21.03 (0.08)	8.39 (0.02)	6.38 (0.22)	3.22 (0.12)	7.5 (0.1)
T-0 Treated	21.15 (0.04)	9.01 (0.01)	7.35 (0.02)	7.53 (0.20)	7.0 (0.1)
T-F Control	20.51 (0.04)	8.41 (0.00)	5.76 (0.03)	2.96 (0.14)	7.0 (0.1)
T-F Treated	20.74 (0.03)	9.04 (0.00)	7.32 (0.01)	7.29 (0.10)	7.0 (0.1)

Live Organisms > 50 µm

	T0 Avg(sd) #/m³	TF Avg(sd) #/m³
Control	160,500 (9,750)	112,500 (5,000)
Treated	17 (7)	1.2 (2)

* Up to four top taxa listed in order of abundance.

*TF Control	*TF Treated
Copepod nauplii Bivalve larvae Spionidae Calanoida (<i>Eurytemora sp.</i>)	Copepoda Polychaeta (Spionidae)

Live Organisms 10 - 50 µm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up these stains.

	T0 #/ml	TF #/ml
Control	4,593 (823)	3,355 (149)
Treatment	0 (0)	0 (0)

Dominant species	Type	Other
<i>Prorocentrum minimum</i>	Dinoflagellate	chain-forming
<i>Thalassiosira sp.</i>	Diatom	
<i>Gyrodinium estuarale</i>	Dinoflagellate	chain-forming
<i>Cyclotella glomerata</i>	Diatom	
<i>Skeletonema costatum</i>	Diatom	chain-forming
<i>Heterocapsa triquetra</i>	Dinoflagellate	
<i>Navicula sp.</i>	Diatom	
<i>Nitzschia sp.</i>	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms.

	T0 µg/l	TF µg/l	RG µg/l
Control	12.33 (0.77)	9.66 (0.16)	NA
Treated	0.04 (0.01)	0.01 (0.00)	NA

RG = after re-growth assay

MDL = 0.56 µg/l

Live Microbes

cfu = colony forming units

<i>E. coli</i>	T0 cfu/100ml	TF cfu/100ml
Control	10.33 (9.29)	2.67 (2.52)
Treated	0 (0)	0 (0)

<i>Enterococci</i>	T0 MPN/100ml	TF MPN/100ml
Control	5.13 (2.05)	8.9 (2.88)
Treated	0.33 (0.58)	0.33 (0.58)

MPN = most probably number estimate determined by the Idexx Enterolert kit.

V. cholerae – No detectable culturable toxigenic *Vibrio cholerae* were detected in any samples during any of the trials.

<i>Heterotrophic Bacteria</i>	T0 cfu/1ml	TF cfu/1ml
Control	2,180 (383)	631.67 (218.68)
Treated	3.5 (6.06)	0 (0)

Toxicity Summaries

Residual Chlorine Analysis – On June 9-10, 2010, MERC assisted Siemens with the testing of their dechlorination system. A 24-hour trial resulted in successful dechlorination of the treated tank using sodium bisulfate (a decline in total chlorine levels from 0.59 ppm to below 0.1 ppm – the EPA discharge standard). Note that using a sodium thiosulfate treatment resulted in a final total chlorine level of 0.22 ppm.

Chronic Toxicity – Ballast water was tested after treatment with a higher concentration of chlorine followed by an in-line de-chlorination. Ballast water was collect in the same manner and location as previous tests. All three species (mysid, fish and algae) were tested for toxicity. There was no statistically significant toxicological effect seen in any of these three test species.

Disinfection By-Products (µg/l)

	Control	Treated	BW	Treated	RDL/M RL
	T0	T0	T0	TF	
Trihalomethanes	0	88.4	0	238.9	0.5
Trichloromethane	< 0.5	< 0.5	< 0.5	< 0.5	0.5
Bromodichloromethane	< 0.5	1.4	< 0.5	2.1	0.5
Dibromochloromethane	< 0.5	15	< 0.5	22.8	0.5
Tribromomethane	< 0.5	72	< 0.5	214	0.5
Haloacetic Acids	0	118.7	0	193.3	1.0
Monochloroacetic acid	< 2.0	< 2.0	< 2.0	< 2.0	2.0
Dichloroacetic acid	< 1.0	< 1.0	< 1.0	< 1.0	1.0
Trichloroacetic acid	< 1.0	< 1.0	< 1.0	< 1.0	1.0
Bromochloroacetic acid	< 1.0	2.5	< 1.0	3.1	1.0
Monobromoacetic acid	< 1.0	2.5	< 1.0	3.8	1.0
Dibromoacetic acid	< 1.0	25	< 1.0	39.4	1.0
Tribromoacetic acid	< 4.0	88.7	< 4.0	147	1.0
Other					
Sodium chlorate	< 10	276/340	< 10/390	233/330	10
Sodium bromate	< 50.0	< 50.0	< 50.0	< 50.0	50.0
Monochloroacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dichloroacetonitrile	<0.5	<0.5	<0.5	<0.5	0.5
Monobromoacetonitrile	<0.5	<0.5	<0.5	<0.5	
Dibromoacetonitrile	<0.5	28	<0.5	29	0.5
Sodium	2630	3170	2620	2880	1.5
NaCl (calculated)	6689	8063	6664	7325	

BW = backwash

RDL = reporting detection limit

MRL = method reporting limit

7.0 Acknowledgments

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16 September 2010

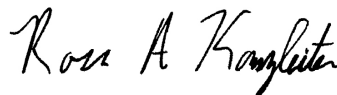
Date



Approved By: Dr. Mario Tamburri
MERC Executive Director

16 September 2010

Date



Approved By: Ross Kanzleiter
MERC Program Coordinator and
Chief Engineer

Final Report

Chronic Toxicity Testing of Siemens Ballast Water Treatment System Year 2 (2010)

Prepared for

Dr. Mario Tamburri

Maritime Environmental Resource Center (MERC)

Chesapeake Biological Laboratory

University of Maryland Center for Environmental Science

PO Box 38/ One Williams Street

Solomons, M.D. 20688

Prepared by

Gregory Ziegler, Dan Fisher, Lance Yonkos and Elizabeth Friedel

University of Maryland

Wye Research and Education Center

P.O. Box 169

Queenstown, MD 21658

July, 2010

Foreword

This toxicity study is part of a larger multiyear project testing the efficacy of ballast water treatment technologies conducted by MERC under the direction of Dr. Mario Tamburri. Toxicity testing was conducted in accordance with International Maritime Organization (IMO) Guidelines to satisfy requirements of Section 5.2 of the Procedures for the Approval of Ballast Water Management Systems.

Abstract

Ballast water treatment technologies are being developed as an important tool in the prevention of invasive species introductions from ballast water discharges. In addition to being effective at killing potential invasive species a ballast water discharge treatment system must be non toxic to receiving water flora and fauna. To satisfy this requirement the IMO has established guidelines for any treatment systems that make use of active substances. The treatment system of Siemens involves the electrochlorination of ballast water to reduce potential invasive species. Toxicity testing was conducted on five samples between 4/27/10 and 6/8/10. Samples were taken from ballast tanks five days after chlorination by the Siemens ballast water treatment system. As required by the IMO, treated ballast discharge was tested for chronic toxicity with three estuarine species; an invertebrate (*Americamysis bahia*), a fish (*Cyprinodon variegatus*) and an algal species (*Isochrysis aff. galbana*).

Results showed that ballast water discharge was not chronically toxic to mysids or Sheepshead Minnows, but was toxic to the algae species *Isochrysis galbana* (T-Iso.) in three out of the five initial ballast water discharge samples.

One additional experiment was conducted on 6/10/10 and consisted of testing ballast water which was previously treated with a higher concentration of chlorine then previously used, followed by an in-line de-chlorination step. Ballast water was collected in the same manner and location as previous tests. All three species (mysid, fish and algae) were tested for toxicity. Although there was no statistically significant toxicological effect seen in any of the test species, there was a non significant decrease in algae concentration at the 56 and 100% concentrations.

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Introduction

All toxicity testing was conducted by the MERC Toxicity Testing Team at the University of Maryland Wye Research and Education Center (WREC). The testing satisfies the requirements of Section 5.2 of the Procedures for the Approval of Ballast Water Management Systems That Make Use of Active Substances (G9) as resolved by the Marine Environmental Protection Committee of the International Maritime Organization (IMO, 2008). This testing is required to ensure the environmental safety of discharging treated ballast water after a holding time of 5 days. Ballast water runs tested for toxicity were SIE-02, SIE-03 and SIE-04. Toxicity tests were conducted on a dilution series of treated ballast water after five days holding time in ballast tanks aboard the Cape Washington. Additional tests were conducted on an untreated sample of Baltimore Harbor water which was held in a duplicate ballast tank and handled in the same manner as the treated ballast water samples. As required by the IMO, treated ballast discharge was tested with three estuarine species; an invertebrate (*Americamysis bahia*), a fish (*Cyprinodon variegatus*) and an algal species (*Isochrysis aff. galbana*). These tests provide survival and sub-lethal (growth) endpoints as well as a true population level endpoint with the algal test. The methods are approved by the USEPA (2002) and the American Society for Testing and Materials (ASTM, 2006). Test method summaries are given in Tables 1 – 3. All water quality requirements were within the acceptable range for all tests. The only deviation from the published methods is the use of a marine algal species (*Isochrysis aff. Galbana*) in place of the fresh water species used in the published guidelines. The species tested is a specific isolate called Tahitian Isochrysis which will be referred to as T-Iso.

Statistical analysis

Data were analyzed using US EPA (2002) and ASTM (2006) statistical procedures. All statistical tests were performed using ToxCalc (TSS, 2006). Cell density for the algal test (T-Iso) test and weight data for the Sheepshead minnow (*Cyprinodon variegatus*) and Mysid (*Americamysis bahia*) were not transformed. All proportional survival data was arc sine square root transformed. Data were analyzed for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Mean values were tested for reductions from the control treatment with a one-tailed Dunnett's test. A p value of 0.05 was used for all hypotheses testing while a p value of 0.01 was used for testing the assumptions of normality and homogeneity of variance. NOEC and LOEC values for average growth rate are reported where toxicity was observed. Regression based point estimates (EC_{50}) were also calculated by linear interpolation in algal tests where there was an effect.

Test acceptability requirements were met for all tests except the requirement for CV% of less than or equal to 20% in control algae tests. Controls in tests SIE-03 (Table 5), SIE-06 (Table 11) and SIE-09 (Table 20) exceeded this with values of 30%, 21.5% and 43.4% for tests SIE-03, 06 and 09, respectively. The control for SIE-06 was deemed close enough to the required CV%, and was not changed for statistical analysis since it was not found to be toxic. SIE-03, on the other hand, had a high CV% of 30 which was driven by one very high cell count. Statistical analysis for SIE-03 was done with the ballast water control which had a CV% of 4.9.

The SIE-09 algae test had a CV of greater than 20% in both the Wye control and the ballast control. The high CV in the Wye control was caused by one unexplained very low density replicate. Statistics were conducted with and without this one control replicate.

Results

Toxicity test results for tests SIE-03 and SIE-05 through SIE-08 are presented in tables 4-18. Results from each test run are grouped to show results for the three tested species together for each test. Results from the mysid tests (Tables 4, 7, 10, 13 and 16) and Sheepshead Minnow tests (Tables 6, 9, 12, 15 and 18) showed no chronically toxic concentrations of ballast water in any of the test runs. Algae tests, on the other hand, identified three out of the five undiluted ballast water discharges as toxic. Samples SIE-03, -05 and -08 (Tables 5, 8 and 17) had LOECs of 100% and NOECs of 56%. All three algae tests with a toxicity response in the undiluted sample (100%) had similar EC_{50} s of 70%, 71% and 78% for SIE-03, -05 and -08, respectively (Table 19).

The coldwater testing of the chlorinated sample without dechlorination resulted in the revealed no statistically significant toxicological effects on any of the species tested (mysids, fish or algae). There was, however, a non-statistically significant decrease in algae at the 56 and 100% ballast water concentrations. There was very low growth in one of the control replicates which decreased the power of the statistics and the ability to statistically distinguish a significant growth reduction. Although there is no explanation for the lower growth in the single control replicate, other than the possibility of some toxic compound in the flask, the elimination of this one replicate in the analysis results in the 100% treatment growth reduction to be statistically significant.

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TSS (Tidepool Scientific Software). 2006. ToxCalc Toxicity Data Analysis Software. Version 5.0.26. Tidepool Scientific Software, McKinleyville, CA.

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Table 1. Summary of the Test Conditions and Test Acceptability Criteria for the Mysid *Americamysis bahia* Larval Survival and Growth Toxicity Test

Test type:	Static renewal
Test duration:	7 d
Temperature:	25°C (± 1°C)
Lighting:	Fluorescent or UV, see above
Photoperiod:	16h light: 8h dark
Test chamber size:	400 mL
Test solution volume:	200 mL
Renewal of test solutions:	Daily
Age of test organisms:	7 d
No. organisms per test chamber:	5
No. replicate chambers per concentration:	8
No. organisms per concentration:	40
Feeding regime:	Feed 150 <24 h old <i>Artemia</i> nauplii daily, half after renewal and half after 8 h.
Test chamber cleaning:	Siphon daily before renewal.
Test chamber aeration:	None, unless DO concentration falls below 4.0 mg/L. Rate should not exceed 100 bubbles/min.
Control water:	Control ballast water or filtered salinity adjusted Wye River water.
Endpoint:	Survival and growth (dry weight).
Test acceptability criterion:	80% or greater survival in controls; average dry weight per surviving organism in control chamber equals or exceeds -0.20 mg.

Table 2. Summary of Test Conditions and Test Acceptability Criteria for the Sheepshead Minnow *Cyprinodon variegatus* Larval Survival and Growth Test

Test type:	Static renewal
Test Duration:	7d
Temperature:	25°C (\pm 1°C)
Lighting:	Fluorescent or UV, see above
Photoperiod:	16h light: 8h dark
Test chamber size:	600-mL glass beaker
Test solution volume:	500 mL
Renewal of test solutions:	Daily
Age of test organisms:	Newly hatched larvae <24 h old
No. larvae per test chamber:	10
No. replicates chambers per concentration:	4
No. larvae per test concentration:	40
Feeding regime:	Artemia nauplii (<24h old). On days 0-2, feed 0.10g wet weight newly hatched (<24h old) brine shrimp nauplii daily. On days 3-6, feed 0.15g wet weight newly hatched (<24h old) brine shrimp nauplii daily.
Cleaning:	Siphon daily, immediately before test solution renewal
Aeration:	None, unless DO concentration falls below 4.0 mg/L. Rate should not exceed 100 bubbles/min
Dilution water:	Filtered Wye River water adjusted to 15‰ with well water or Crystal Sea Bioassay Mix [®]
Test concentrations:	5 and control
Dilution factor:	0.5
Endpoint:	Survival and growth (dry weight)
Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chamber equals or exceeds 0.60 mg

Table 3. Summary of Test Conditions and Test Acceptability Criteria for the Alga *Isochrysis aff. galbana* (T-Iso) Chronic Growth Test

Test type:	Static non-renewal (required)
Test duration:	72-96h
Temperature:	25°C (± 1°C)
Lighting:	Fluorescent
Photoperiod:	Continuous illumination
Test chamber size:	125-mL Erlenmeyer flasks
Test solution volume:	50 mL
Renewal of test solutions:	None
Age of test organisms:	4 to 7d in log growth phase
Initial cell density in test chambers:	10,000 cells/mL
Shaking rate:	100 rpm continuous on a mechanical shaker
Aeration:	None
Nutrient Solution:	f/2 algal culture medium nutrients added to each replicate.
Dilution water:	Wye River water
Test concentrations:	Treated ballast water dilutions of 100, 56,32,18,and 10% plus Wye River control and 100% Baltimore Harbor sample from untreated ballast tank.
Dilution series:	0.56
Endpoint:	Growth (cell counts)
Test acceptability criterion:	Mean cell density of at least 3×10^5 cells/mL in the controls; and variability (CV%) among control replicates less than or equal to 20%

Table 4. Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-03-10 (4/27/10-5/4/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.		# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control	A	5	0.20	77 (16.7))	0.171 (.030)
	B	3	0.18		
	C	3	0.14		
	D	4	0.19		
	E	5	0.22		
	F	4	0.14		
	G	4	0.15		
	H	3	0.15		
32%	A	4	0.20	82 (16.7)	0.175 (0.0359)
	B	5	0.21		
	C	3	0.13		
	D	5	0.21		
	E	3	0.13		
	F	5	0.18		
	G	4	0.20		
	H	4	0.14		
56%	A	5	0.24	82 (16.7)	0.225 (0.0374)
	B	4	0.24		
	C	4	0.26		
	D	5	0.26		
	E	5	0.25		
	F	4	0.21		
	G	3	0.17		
	H	3	0.17		
100%	A	5	0.25	90 (15.1)	0.237 (0.0507)
	B	4	0.23		
	C	5	0.26		
	D	5	0.34		
	E	3	0.17		
	F	4	0.22		
	G	5	0.20		
	H	5	0.23		
Untreated	A	3	0.16	70 (23.9)	0.239 (0.0710)
	B	2	0.16		
	C	2	0.15		
	D	4	0.27		
	E	4	0.28		
	F	3	0.20		
	G	5	0.30		
	H	5	0.33		

Table 5. Final cell density data for the algae T-Iso exposed to ballast water dilution series. Test results (96-h) from test SIE-03-10 conducted from 4/27/10-4/31/10. An * indicates a treatment significantly less than control ($\alpha=0.05$).

Concentration	Replicate Mean Count				Conc. Mean Count	Mean Density ($\times 10^6/\text{ml}$)	CV%
	A	B	C	D			
Control	139	102	68	88	99	5.1	30
32%	51	87	76	72	71	3.6	21
56%	84	86	72	39	70	3.6	30
* 100%	11	7	10	4	8	0.41	41
Untreated	73	73	79	78	76	3.8	4.5

Table 6. Ballast water *Cyprinodon variegatus* 7-d short-term chronic toxicity test results from test SIE-03-10 (4/27/10-5/4/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Larvae	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	10	0.61	100	0.62 (0.031)
B	11	0.65		
C	10	0.65		
D	10	0.59		
10% A	10	0.64	97 (5)	0.64 (0.014)
B	10	0.65		
C	10	0.62		
D	9	0.65		
18% A	10	0.70	100	0.65 (0.031)
B	10	0.65		
C	10	0.64		
D	10	0.63		
32% A	10	0.66	92.5 (15)	0.65 (0.060)
B	10	0.68		
C	7	0.56		
D	11	0.69		
56% A	10	0.72	100	0.71 (0.017)
B	10	0.73		
C	10	0.72		
D	10	0.69		
100% A	9	0.64	95 (4.7)	0.65 (0.008)
B	9	0.66		
C	10	0.65		
D	10	0.65		
Untreated A	10	0.66	97.5 (5)	0.65 (0.015)
B	9	0.63		
C	10	0.66		
D	10	0.66		

Table 7. Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-05-10 (5/18/10-5/25/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	5	0.28	90 (10.7)	0.224 (0.0333)
B	4	0.24		
C	4	0.23		
D	5	0.20		
E	4	0.19		
F	5	0.25		
G	4	0.18		
H	5	0.22		
10% A	4	0.20	90 (10.7)	0.210 (0.0302)
B	4	0.19		
C	5	0.21		
D	5	0.21		
E	5	0.21		
F	5	0.24		
G	4	0.16		
H	4	0.26		
18% A	5	0.22	97.5 (7.1)	0.234 (0.0217)
B	5	0.27		
C	5	0.24		
D	5	0.23		
E	4	0.23		
F	5	0.21		
G	5	0.27		
H	5	0.24		
32% A	4	0.18	80 (21.4)	0.198 (0.0473)
B	5	0.24		
C	5	0.21		
D	4	0.18		
E	2	0.11		
F	5	0.27		
G	3	0.19		
H	4	0.21		

Table 7 (continued). Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-05-10 (5/18/10 - 5/25/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
56% A	4	0.16	92 (10.3)	0.207 (0.0456)
B	5	0.17		
C	5	0.22		
D	4	0.15		
E	5	0.29		
F	4	0.22		
G	5	0.23		
H	5	0.22		
100% A	5	0.22	90 (15.1)	0.211 (0.0430)
B	3	0.14		
C	4	0.18		
D	4	0.20		
E	5	0.23		
F	5	0.21		
G	5	0.22		
H	5	0.29		
Untreated A	3	0.18	80 (18.5)	0.221 (0.0295)
B	5	0.21		
C	5	0.21		
D	4	0.21		
E	4	0.27		
F	4	0.25		
G	3	0.20		
H	5	0.24		

Table 8. Final cell density data for the algae T-Iso exposed to ballast water dilution series. Test results (72-h) from test SIE-05-10 conducted from 5/18/10 - 5/21/10. An * indicates a treatment significantly less than control ($\alpha=0.05$).

Concentration	Replicate Mean Count				Conc. Mean Count	Mean Density (x10 ⁶ /ml)	CV%
	A	B	C	D			
Control	68	58	65	80	68	3.4	14.5
10%	68	71	68	72	70	3.6	2.7
18%	66	58	54	103	70	3.6	30.4
32%	57	68	69	71	66	3.4	9.4
56%	43	51	59	43	49	2.5	15.3
*100%	7	7	4	6	6	0.31	21.4
Untreated	35	37	41	37	37	1.9	8.6

Table 9. Ballast water *Cyprinodon variegatus* 7-d short-term chronic toxicity test results from test SIE-05-10 (5/18/10 - 5/25/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Larvae	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	10	0.47	100	0.53 (0.037)
B	10	0.54		
C	10	0.55		
D	10	0.56		
10% A	10	0.64	100	0.60 (0.033)
B	10	0.60		
C	10	0.58		
D	10	0.56		
18% A	10	0.67	100	0.67 (0.019)
B	10	0.64		
C	10	0.68		
D	10	0.67		
32% A	10	0.68	100	0.66 (0.013)
B	10	0.66		
C	10	0.67		
D	10	0.65		
56% A	10	0.69	100	0.69 (0.016)
B	10	0.67		
C	10	0.70		
D	10	0.68		
100% A	10	0.61	100	0.63 (0.029)
B	10	0.64		
C	10	0.61		
D	10	0.67		
Untreated A	10	0.56	100	0.58 (0.018)
B	10	0.59		
C	10	0.57		
D	10	0.60		

Table 10. Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-06-10 (5/25/10 - 6/1/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.		# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control	A	5	0.26	97 (7.1)	0.252 (0.0167)
	B	4	0.23		
	C	5	0.23		
	D	5	0.26		
	E	5	0.25		
	F	5	0.28		
	G	5	0.25		
	H	5	0.26		
10%	A	5	0.22	97 (7.1)	0.232 (0.2605)
	B	5	0.24		
	C	5	0.22		
	D	5	0.21		
	E	5	0.29		
	F	5	0.24		
	G	4	0.21		
	H	5	0.23		
18%	A	5	0.26	90 (15.1)	0.239 (0.0242)
	B	4	0.25		
	C	5	0.28		
	D	3	0.23		
	E	5	0.21		
	F	5	0.21		
	G	5	0.24		
	H	4	0.23		
32%	A	4	0.18	85 (9.2)	0.205 (0.0288)
	B	4	0.20		
	C	4	0.17		
	D	4	0.22		
	E	5	0.24		
	F	4	0.23		
	G	4	0.17		
	H	5	0.23		
56%	A	5	0.24	95 (14.1)	0.221 (0.0318)
	B	3	0.20		
	C	5	0.20		
	D	5	0.20		
	E	5	0.23		
	F	5	0.20		
	G	5	0.29		
	H	5	0.21		

Table 10 (continued). Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-06-10 (5/25/10 - 6/1/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
100% A	4	0.17	95 (9.2)	0.250 (0.0385)
B	5	0.25		
C	5	0.28		
D	5	0.27		
E	5	0.25		
F	4	0.22		
G	5	0.28		
H	5	0.28		
Untreated A	5	0.25	90 (15.1)	0.284 (0.0311)
B	4	0.26		
C	4	0.25		
D	3	0.31		
E	5	0.32		
F	5	0.31		
G	5	0.26		
H	5	0.31		

Table 11. Final cell density data for the algae T-Iso exposed to ballast water dilution series. Test results (72-h) from test SIE-06-10 conducted from 5/25/10 – 5/28/10. An * indicates a treatment significantly less than control ($\alpha=0.05$).

Concentration	Replicate Mean Count				Conc. Mean Count	Mean Density ($\times 10^6/\text{ml}$)	CV%
	A	B	C	D			
Control	86	62	91	58	74	3.8	21.5
10%	41	89	29	70	57	2.9	46.9
18%	58	86	113	58	79	4.0	33.1
32%	62	51	65	71	62	3.1	13.2
56%	63	77	79	83	75	3.8	11.4
100%	86	57	68	76	72	3.7	17.3
Untreated	77	56	48	44	56	28.2	26.9

Table 12. Ballast water *Cyprinidon variegates* 7-d short-term chronic toxicity test results from test SIE-06-10 (5/25/10 - 6/1/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Larvae	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	9	0.43	97 (5)	0.48 (0.036)
B	10	0.50		
C	10	0.51		
D	10	0.49		
10% A	10	0.43	100	0.48 (0.034)
B	10	0.50		
C	10	0.51		
D	10	0.49		
18% A	10	0.55	100	0.57 (0.027)
B	10	0.58		
C	10	0.60		
D	10	0.54		
32% A	9	0.61	97 (5)	0.59 (0.027)
B	10	0.60		
C	10	0.60		
D	10	0.55		
56% A	10	0.58	100	0.57 (0.017)
B	10	0.58		
C	10	0.59		
D	10	0.55		
100% A	10	0.53	100	0.56 (0.022)
B	10	0.56		
C	10	0.57		
D	10	0.58		
Untreated A	10	0.52	100	0.49 (0.021)
B	10	0.47		
C	10	0.49		
D	10	0.49		

Table 13. Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-07-10 (6/1/10 - 6/8/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth	
Control	A	5	0.27	95 (9.2)	0.246 (0.0297)
	B	5	0.24		
	C	5	0.21		
	D	5	0.22		
	E	5	0.28		
	F	5	0.27		
	G	4	0.21		
	H	4	0.27		
10%	A	5	0.29	100	0.291 (0.0394)
	B	5	0.38		
	C	5	0.25		
	D	5	0.29		
	E	5	0.28		
	F	5	0.28		
	G	5	0.26		
	H	5	0.30		
18%	A	4	0.20	95 (9.2)	0.26 (0.0342)
	B	4	0.24		
	C	5	0.26		
	D	5	0.32		
	E	5	0.28		
	F	5	0.27		
	G	5	0.25		
	H	5	0.26		
32%	A	5	0.21	82 (12.8)	0.234 (0.045)
	B	3	0.15		
	C	4	0.22		
	D	4	0.25		
	E	4	0.22		
	F	4	0.30		
	G	5	0.27		
	H	4	0.25		
56%	A	5	0.24	97.5 (7.1)	0.272 (0.0167)
	B	4	0.28		
	C	5	0.29		
	D	5	0.26		
	E	5	0.27		
	F	5	0.28		
	G	5	0.27		
	H	5	0.29		

Table 13 (continued). Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-07-10 (6/1/10 - 6/8/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
100% A	5	0.24	95 (9.2)	0.229 (0.0505)
B	5	0.25		
C	5	0.27		
D	5	0.13		
E	5	0.29		
F	5	0.25		
G	4	0.20		
H	4	0.20		
Untreated A	5	0.28	95 (14.1)	0.301 (0.0690)
B	5	0.27		
C	5	0.35		
D	5	0.21		
E	5	0.29		
F	5	0.26		
G	5	0.31		
H	3	0.44		

Table 14. Final cell density data for the algae T-Iso exposed to ballast water dilution series. Test results (72-h) from test SIE-07-10 conducted from 6/1/10 – 6/4/10. An * indicates a treatment significantly less than control ($\alpha=0.05$).

Concentration	Replicate Mean Count				Conc. Mean Count	Mean Density ($\times 10^6/\text{ml}$)	CV%
	A	B	C	D			
Control	64	68	82	55	67	3.4	16.8
56%	67	58	65	77	67	3.1	7.6
100%	59	56	52	62	57	2.9	8.5
Untreated	56	47	54	54	53	3.4	16.8

Table 15. Ballast water *Cyprinodon variegatus* 7-d short-term chronic toxicity test results from test SIE-07-10 (6/1/10 - 6/8/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Larvae	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	10	0.58	100	0.68 (0.075)
B	10	0.68		
C	10	0.74		
D	10	0.74		
10% A	10	0.76	100	0.78 (0.016)
B	10	0.80		
C	10	0.78		
D	10	0.78		
18% A	10	0.87	97 (5)	0.83 (0.024)
B	10	0.83		
C	10	0.82		
D	9	0.82		
32% A	10	0.80	97 (5)	0.81 (0.013)
B	9	0.81		
C	10	0.82		
D	10	0.83		
56% A	10	0.80	97 (5)	0.79 (0.021)
B	9	0.77		
C	10	0.81		
D	10	0.77		
100% A	10	0.62	100	0.68 (0.053)
B	10	0.67		
C	10	0.68		
D	10	0.75		
Untreated A	10	0.60	100	0.58 (0.041)
B	10	0.61		
C	10	0.52		
D	10	0.59		

Table 16. Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-08-10 (6/8/10 - 6/15/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control	A	4	0.19	90 (10.7) 0.235 (0.0220)
	B	5	0.26	
	C	4	0.24	
	D	4	0.25	
	E	5	0.24	
	F	4	0.22	
	G	5	0.25	
	H	5	0.23	
56%	A	4	0.20	92 (14.8) 0.246 (0.0665)
	B	5	0.28	
	C	5	0.28	
	D	5	0.26	
	E	3	0.10	
	F	5	0.26	
	G	5	0.29	
	H	5	0.30	
100%	A	4	0.21	95 (9.2) 0.221 (0.0203)
	B	4	0.20	
	C	5	0.23	
	D	5	0.24	
	E	5	0.19	
	F	5	0.23	
	G	5	0.22	
	H	4	0.25	
Untreated	A	5	0.25	92 (10.3) 0.231 (0.0452)
	B	5	0.26	
	C	5	0.23	
	D	5	0.21	
	E	4	0.19	
	F	4	0.18	
	G	5	0.32	
	H	4	0.21	

Table 17. Final cell density data for the algae T-Iso exposed to ballast water dilution series. Test results (72-h) from test SIE-08-10 conducted from 6/8/10 – 6/11/10. An * indicates a treatment significantly less than control ($\alpha=0.05$).

Concentration	Replicate Mean Count				Conc. Mean Count	Mean Density ($\times 10^6/\text{ml}$)	CV%
	A	B	C	D			
Control	159	220	239	261	220	2.2	20.9
32%	229	141	299	313	245	2.5	33.5
56%	159	226	185	142	178	1.8	21.7
* 100%	52	46	54	63	54	0.5	12.1
Untreated	178	188	188	182	184	1.9	2.7

Table 18. Ballast water *Cyprinodon variegatus* 7-d short-term chronic toxicity test results from test SIE-08-10 (6/8/10 - 6/15/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Larvae	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	10	0.50	100	0.54 (0.034)
B	10	0.54		
C	10	0.56		
D	10	0.58		
56% A	10	0.61	100	0.59 (0.016)
B	10	0.57		
C	10	0.59		
D	10	0.59		
100% A	10	0.64	100	0.61 (0.024)
B	10	0.63		
C	10	0.60		
D	10	0.59		
Untreated A	9	0.55	97 (5)	0.58 (0.031)
B	10	0.60		
C	10	0.62		
D	10	0.57		

Table 19. Table 16. Ballast water *Americamysis bahia* 7-d short-term chronic toxicity test results from test SIE-09-10 (6/10/10 - 6/17/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Mysids	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	4	0.07	67 (23.7)	0.11 (0.040)
B	2	0.06		
C	2	0.07		
D	4	0.19		
E	2	0.12		
F	5	0.16		
G	4	0.15		
H	4	0.11		
56% A	4	0.14	75 (17.7)	0.11 (0.041)
B	4	0.11		
C	4	0.12		
D	4	0.12		
E	4	0.09		
F	3	0.08		
G	2	0.06		
H	5	0.19		
100% A	4	0.10	65 (20.7)	0.10 (0.041)
B	4	0.16		
C	2	0.10		
D	4	0.10		
E	4	0.13		
F	4	0.13		
G	2	0.09		
H	2	0.09		
Not Dechlorinated A	4	0.16	82 (12.8)	0.13 (0.023)
B	5	0.14		
C	3	0.10		
D	4	0.12		
E	5	0.11		
F	4	0.16		
G	4	0.13		
H	4	0.12		

Table 20. Final cell density data for the algae T-Iso exposed to ballast water dilution series. Test results (72-h) from test SIE-09-10 conducted from 6/10/10 – 6/17/10. An * indicates a treatment significantly less than control ($\alpha=0.05$).

Concentration	Replicate Mean Count			Conc. Mean Count	Mean Density ($\times 10^6/\text{ml}$)	CV%
	A	B	C			
Control	136	147	155	128	6.2	43.41
32%	240	189	151	193	10.0	17.7
56%	74	126	118	106	5.4	25.9
100%	81	50	103	78	4.1	27.2
Non-dechlor	0	0	0	0	0	0

Table 21. Ballast water *Cyprinodon variegatus* 7-d short-term chronic toxicity test results from test SIE-09-10 (6/11/10 - 6/18/10). An * indicates a treatment significantly less than control ($\alpha=0.05$).

Treatment rep.	# Surviving Larvae	Mean Rep. Growth Dry Wt.(mg)/Organism	Mean (SD) Treatment % Survival	Mean (SD) Treatment growth
Control A	10	0.48	100	0.47 (0.008)
B	10	0.47		
C	10	0.48		
D	10	0.50		
56% A	10	0.52	97.5 (5)	0.50 (0.032)
B	9	0.54		
C	10	0.47		
D	10	0.48		
100% A	10	0.50	97.5 (5)	0.51 (0.047)
B	10	0.49		
C	10	0.48		
D	9	0.58		
Untreated A	9	0.58	97.5 (5)	0.56 (0.029)
B	10	0.59		
C	10	0.55		
D	10	0.52		

Table 22. LOEC, NOEC and EC values for all algal (T-Iso) tests (SIE-03 and SIE-05 through -09). LOEC and NOECs based on average growth rate. EC₅₀ values determined by linear interpolation and presented with 95% confidence intervals.

Sample ID (length)	NOEC %	LOEC %	EC ₅₀ % (95% CI)
SIE-03 (96-h)*	56	100	70.4 (37.7-84.8)
SIE -05 (72-h) **	56	100	70.6 (61.3-76.8)
SIE -06 (72-h) **	100	ND	ND
SIE -07 (72-h) **	100	ND	ND
SIE -08 (72-h) **	56	100	77.8 (60.4-89.8)
SIE-09 (72-h) ***	100	ND	ND

ND (not determined)

* Exceptable test according to EPA test protocols (EPA method 1003.0).

** Exceptable test according to OECD test protocols (OECD 201).

*** Unacceptable test due to high CV% in the controls (See pg 2 for explanation).

Table 23. LOEC and NOEC values for all mysid and Sheepshead minnow tests (SIE-03 and SIE-05 through -09).

	Mysid		Fish	
Sample ID	NOEC %	LOEC %	NOEC %	LOEC %
SIE-03	100	ND	100	ND
SIE -05	100	ND	100	ND
SIE -06	100	ND	100	ND
SIE -07	100	ND	100	ND
SIE -08	100	ND	100	ND
SIE -09	100	ND	100	ND

ND (not determined)